LAY ABSTRACT

The mammary gland is a tree-like structure formed by hollow ducts. These ducts are lined by epithelial cells, and they finish in lobular structures known as terminal end buds (TEBs). Stem cells reside in the TEBs during mammary gland development, being responsible for ductal growth. It has been shown that the use of carcinogens (cancer-causing agents) produces transformation in the TEBs. It has also been established that markers for stem cells appear at higher concentrations in human breast cancer. Therefore, it seems that stem cells play an important role in breast cancer onset and development. To understand the biology of stem cells and their role in the malignant transformations occurring in cancer it is essential to determine their three-dimensional distribution.

We will use a label retention technique to identify stem cells. In this method, a certain label (Bromodeoxyuridine, BrdU) is pumped inside the nuclei of the cells for some time. Then the cells lose the label progressively as they replicate. The more times a cell divides, the more label it loses. Stem cells replicate infrequently, and therefore retain the label inside their nuclei. Later on, using immunofluorescence techniques, we will add a fluorescent molecule to the label, so that only the cells that retained it will fluoresce.

We will use the label retention technique in entire mouse mammary glands. The glands will then be sliced into thin sections, and each of these sections will be imaged using a computer-assisted 3D microscopy system that we have developed. Then we will create three-dimensional models of these mammary glands, reconstructing them from the previously acquired images. These models will show the distribution of the labels in the three-dimensional mammary glands.

It has been shown for some organs (e.g. mouse skin and murine intestine) that the stem cells give raise to organized structures or functional units with certain common characteristics. These functional units include also the progeny of the stem cells that give raise to them. Our hypothesis is that in the mouse mammary gland there is also such a structure. We aim at determining this by using 3D models of the mouse mammary glands as the initial data. We will detect all the fluorescent signals (label-retaining cells, LRCs) in the different sections of each gland using image analysis techniques. Then, we will develop computer software tools to determine the three-dimensional distribution of those signals in a mammary gland (3D spatial pattern analysis). We want to know whether the three-dimensional distribution of the LRCs is random or not, and if it is not, we want to determine which is that distribution and how it relates to the ones observed in other organs.

Finally we want to repeat these experiments with mice genetically engineered to overexpress a certain gene, *c-neu*, whose human counterpart is overexpressed in more than 30% of human breast cancer cases. These mice develop tumors that grow slowly, and that allows for the study of cancer progression. Thus, we will investigate the changes that LRCs and their three-dimensional distribution undergo in tumor onset and development, and their contribution to these events.

The purpose of the work outlined in this abstract is to develop novel image analysis techniques to study the three-dimensional organization of stem cells within the mammary gland of mice and their transformation in breast cancer. Modulation of stem cell behavior holds exceptional promise for a new prophylactic approach for controlling cancer risk in general and breast cancer risk in particular. The three-dimensional characterization of the distribution of stem cells and their environment will be an important step towards understanding stem cell behavior and, therefore, towards new modalities of cancer prevention and therapy.